CHAPTER 5

5. DISCUSSION

The unscientific extraction of coal in the unorganized sector is going on since long time in Tirap Colliery of Makum Coalfield of Assam. Mining operation has resulted in drastic changes in the soil and water quality and the area affected by coal mining is increasing day by day. As the problem has become acute in mining and adjacent areas of Tirap Colliery of Upper Assam, so it becomes necessary to initiate regreening/rehabilitation programs to minimize the effects of post mining operation in Tirap Colliery. The present investigation was designed to assess and standardize selection of most suitable treatment/treatment combination for restoration and revegetation/regreening of Tirap Colliery. In this piece of research work emphasis has been given on isolation, identification and characterization of native microorganisms along with selection of suitable treatment method for the regreening purpose and to restore the degraded ecosystem by introducing entrapping plants to the polluted sites.

5.1 SOIL ANALYSIS

In the present investigation, the soil samples collected from the study area were studied for textural analysis and physico-chemical properties. The soil textural analysis (Table 5) of collected samples showed that the average highest sand % (89.4) was recorded in sample no. S5 while the lowest % (84.4) was
recorded in sample no. S4. The average percentage of clay, silt and sand varies from 5.81 – 10.61, 3.99 – 7.42 and 84.4 – 89.40 respectively and sand fraction appeared to be predominant in all samples. Dollhoph et al. (1981) suggested that when sand content became more than 70% the coalmine spoils retain insufficient water for plant production and thus produce unfavorable conditions for both the microbes and plants. Therefore high sand content in the soil samples of Tirap Colliery might be a reason of very sparse vegetation in degraded mine spoil areas. The sandy nature of mine wastes aggravates the situation further for vegetation establishment by developing low infiltration rates and water retention. Since the progress of natural vegetation process is very slow on mine spoils, selective plantation of suitable native species is desired in most cases.

Soil physico-chemical characteristics (Table 6) viz. water holding capacity (WHC), soil moisture content (SMC), pH, conductivity, available phosphorus, organic carbon, soil organic matter and total nitrogen content were determined from collected overburden soils of Tirap Colliery in triplicate. From table 6, it was observed that the WHC of soil samples were below 60%. The highest (59.49%) and the lowest (34.36%) WHC were recorded in S7 and S3 respectively. The highest (29.7%) and the lowest (8.04%) SMC was recorded in S7 and S1. From the results, it is clear that the WHC and SMC in soil samples are low due to its sandy soil texture. Doubleday et al. (1977) reported that salinity, poor water holding capacity, acidity and reduced nutrients availability are the major problems in coal mine spoils. The pH of the most of the soil samples ranged from 3.25 – 4.75 which confirmed that the soil pH is very low and thus
highly acidic in nature. Similar reports were confirmed by Johnson and Bradshaw (1977) they stated that pH is a major determinant in controlling plant growth on impoverished lands such as mine spoils. They reported pH of 3.5, which indicates the acute acidity of the soil. Hazarika et al. (2004) reported that the coalmine spoils are highly acidic (pH 2.8 – 6.2) due to oxidation of sulphur to sulphuric acid. Power (1978) considers soil physico-chemical characteristics like texture, pH, electrical conductivity, soluble Ca, Mg, Na, B, cation exchange capacity, exchangeable cations, gypsum and calcium carbonate equivalents as being crucial to the prediction of plant growth potential of mine overburdens with WHC and infiltration rates as the other important variables. Amount of organic carbon and organic matter differs considerably among the collected soil samples of Tirap Colliery. The results obtained showed lower amount of nutrients (available P, organic matter and total nitrogen) that may be one of the major factor, which affects the vegetation process in Tirap Colliery. All soil samples contain some organic matter, but relatively few contain enough to be classified as organic soils. Soil are classified as low (0.5), medium (0.5 – 0.750 and high organic carbon content (more than 0.75). All soil samples come under the class range of high organic carbon content. The maximum percentage of organic carbon (5.98%) and matter (9.87%) was recorded in sample S2 and S3. Lower amounts of available phosphorus and nitrogen was recorded in all the collected soil samples. William (1975) stated similar report of lower amounts of nitrogen and phosphorus in mine spoils. He stated that nitrogen and phosphorus are the major limiting nutrient in the coal mine spoils. The results obtained are in
agreement with the investigations of Power and Bennet (1977), Dutta and Agarwal (2005), Richards et al. (1996) and Jha and Singh (1990). They also considered reduced nutrient availability as a major problem that affected the vegetation process in various mine spoils. Soil fertility is a major factor regulating plant growth. The physical factors which limit plant establishment and survival include high temperature, moisture stress (Richardson 1975), soil particle size (Down 1974) and compaction (Hall 1957, Richardson 1975). However Bradshaw et al. (1975) and Bell and Ungar (1981) found high temperature and low moisture of surface coalmine spoils to be important factors limiting plant growth.

5.2 WATER ANALYSIS

It was observed (Table 7) that the water samples were collected from three different sources. The pH of water samples W₁ (2.98) and W₂ (4.37) were very low which confirms the acute acidity of water samples in the operating and neighboring areas. However, the pH of Tirap River, water sample (W₃) was found within the range of permissible limit when compared with the limits of IS: 10500. The highest amount of sulphate was recorded in water sample W₁ (1317.2 mg/L) followed by W₂ (791.4 mg/L). The amount of sulphate in water sample W₁ and W₂ exceeded the maximum limits (IS: 10500) while the water sample (W₃) showed a very low concentration of sulphate ion (37.8 mg/L) and was found within the prescribed limits of IS: 10500. From the result it is confirmed that the low pH of water in the study area is due to presence of free H₂SO₄ &
Regreening of degraded soil of Tirap Colliery of Makum Coalfield of Assam, India

ferric sulphate along with other salts. The high concentration of sulphate is mainly due to presence of iron sulphide in coal and rocks and its reaction with water and oxygen. Dowarah et al. (2009) confirmed that due to the presence of high sulphur content (2 – 12%), the mine OB of the NE collieries is highly acidic (pH 2.0–3.0). Similar results were earlier confirmed by Chadwick (1973), Bhattacharya et al. (1999), Hazarika et al. (2004) and Rawat et al. (2006). Bhattacharya et al. (1999) reported low pH and conductivity in water samples of Makum coalfield of Assam. Rawat et al. (2006) reported high sulphate ranging up to 1500 ppm and iron content up to 40 ppm in water samples of Margherita of North Eastern Coalfield. They confirmed the presence of iron oxidizing, sulphur oxidizing and iron sulphur oxidizing bacteria in mine water. These bacteria accelerate the sulphur leaching rate from coal and are indigenous to mine drainages. Chadwick (1973) reported similar kind of problems due to oxidation of iron pyrites which ultimately results in extreme acidity in the coalmine soil and consequently, ecological succession takes longer time.

5.3 HEAVY METAL ESTIMATION

The collected water and soil samples were analyzed for the presence of heavy metal viz; Cadmium (Cd), Copper (Cu), Zinc (Zn) and Lead (Pb). From Table 8, it was observed that the Lead (Pb) was not detected (ND) in any of the water samples. However, other metals viz Cadmium (Cd), Copper (Cu) and Zinc (Zn) were detected in varied concentration. Copper (Cu) and Zinc (Zn) were found within the prescribed limits when compared with the limits of IS: 10500.
Higher concentration of Cadmium (Cd) was detected in both the water samples $W_1$ and $W_2$ and both exceeded the maximum limits when compared with the limits of IS: 10500. No trace of Cadmium (Cd) was detected in water sample ($W_3$) of Tirap River. It was observed (Table 9) that Lead (Pb) was not detected in any of the soil samples. The metal Cadmium (Cd) and Zinc (Zn) were detected in varied concentrations in all the soil samples while Copper (Cu) was detected only in sample no. $S_6$, $S_7$ and $S_{10}$ respectively. The highest concentration of Cadmium (Cd) and Zinc (Zn) was recorded in sample no. $S_2$.

From the result it is clear that the soil samples are contaminated with high concentration of Cadmium (Cd) and Zinc while other metals were present in very low concentrations. Wali (1975) reported that Water-soluble Boron, Cu, Fe, and Li. Sr & Zn contents were greater in mine spoils however some of these trace metals may cause serious health hazards to the mankind even in very low concentration.

### 5.4 MICROBIAL POPULATION IN THE OBD SOIL OF TIRAP COLLIERY

The total bacterial and fungal counts in the soil of Tirap Colliery were studied and it was found that the old OBD soil showed higher population of bacterial & fungal counts ($103 \times 10^4$ and $61 \times 10^4$ per gm of soil) than the new OBD soil ($70 \times 10^4$ and $40 \times 10^4$ per gm of soil) of Tirap Colliery (Table 10). From the table it is clear that the population of bacteria was higher than the fungal population. Frequez & Lindeman (1982) also reported low diversity of
fungi in the coalmine overburden soil. Table 11 reveals the population dynamics of certain groups of bacteria. The population of gram (-)ve bacteria \((0.98 \times 10^6)\) was highest in number followed by phosphate solubilizing bacteria \((0.43 \times 10^6)\), denitrifying bacteria \((0.36 \times 10^6)\), asymbiotic \(N_2\) fixing bacteria \((0.23 \times 10^6)\) and nitrifying bacteria \((0.21 \times 10^6)\) in per gm of overburden soil of Tirap Colliery.

5.5 FLORAL SPECIES OF TIRAP COLLIERY

The vegetation patterns including both naturally occurring plants and introduced plants in OBD soil of Tirap Colliery were studied and recorded in Table 12. Most of the naturally growing plants belong to Poaceae Family which includes plants viz; \(C. dactylon\), \(T. maxima\), \(A. aciculatus\) and \(A. compressus\). However plants like \(M. pudica\) and \(C. asiatica\) of family Mimosaceae and Apiaceae are also growing profusely in the degraded soil of Tirap Colliery. The other plant species introduced by the coalmine authorities in the Tirap Colliery for the regreening/rehabilitation of degraded soil includes \(Acacia\) sp., \(Cassia\) sp., \(Gamelina\) sp., \(Jatropha\) sp., \(Bambusa\) sp. (Fig. 10).

5.6 CHARACTERIZATION AND IDENTIFICATION OF FUNGAL ISOLATES

The occurrence of AM Fungal spores in the plant species growing in degraded soil of Tirap Colliery was studied and recorded in Table 13. The numbers of spores were dominant in plants belonging to Poaceae Family. The
AM Fungal spores isolated and identified were belong to *Glomus* and *Acaulospora* Family. The *Glomus* species was dominant and recorded highest number of spores in *C. dactylon* of Poaceae Family. Kumar *et al.* (2003) surveyed 79 plant species belonging to 30 families and found six families namely Poaceae (13 species), Fabaceae (7 species), Mimosaceae (5 species), Asteraceae (6 species), Moraceae (4 species) and Amaranthaceae (4 species) possessed most frequent VAM association. They reported high level of VAM colonization in 29 plants including *A. nilotica, Butea monosperma, Calotropis procera, C. dactylon, D. sissoo, Ficus benghalensis, Grevilla pteridofolia, Holarrhena antidysentrica* etc.

The Table 14 represents the characterization and identification of the fungal isolates. A total of 8 genera of fungi viz; *Aspergillus* sp., *Penicillium* sp., *Curvularia* sp., *Trichoderma* sp., *Fusarium* sp., *Mucor* sp., *Mycelia* sp. and *Rhizopus* sp. were identified along with very few species of AM Fungi viz *Glomus* sp. and *Acaulospora* sp. (Fig. 11(a) to Fig. 11(k)). Noordwijk and Hairiah (1986) worked on the occurrence of AM Fungi and found that certain species of AM Fungi were adversely affected by low pH of the soil.

### 5.7 CHARACTERIZATION AND IDENTIFICATION OF THE BACTERIAL ISOLATES

The bacterial grouping and identification was done on the basis of microscopic observations, characterization and biochemical tests. A total of 13 groups were identified and presented in Table 15, while Table 16 presented the
characterization of the isolated bacterial isolates by different biochemical tests where genus *Rhizobia* and *Pseudomonas* were identified along with other unidentified groups. The identified bacterial isolates were studied for their growth on different pH and Temperature (Fig. 14(a) and 14(b)). The bacterial isolates BDT 4, 5, 12 and 13 showed good growth at pH 7 and 8. However, it was found that some of the bacterial isolates BDT 2, 3, 5, 6 and 13 were also able to grow fairly at pH 4.5 while BDT 2, 3, 4, 5, 6, 8, 10, 12 and 13 showed better growth at Temperature 25 °C and 30 °C.

### 5.8 POTENTIAL MICROORGANISMS WITH PGPRs ACTIVITY

Table 17 represents the isolated potential microorganisms and inoculated microorganisms. Most of the potential microorganisms were entrapped by the test plants (primary entrapped plants). While some of the important potential microorganisms found in the degraded soil of Tirap Colliery were inoculated to the secondary plants as booster dose. So, the microorganism’s *viz.* *Rhizobium* sp., *Pseudomonas* sp. and *G. mosseae* were inoculated directly as inoculum into the soil during the plant experimental study for the regreening of the polluted / degraded sites of Tirap Colliery.

### 5.9 PLANTATION STUDY

In this study, two varieties of test plants *viz.* *M. atropurpureum* (*V*₁) and *C. cajan* (*V*₂) were studied for the overall survival percentage, growth (plant height), pod yield, nodule production and nutrient status (available P, nitrogen,
organic carbon and organic matter) of the soil under different treatments /
treatment combinations and time period.

5.9.1 Survivability of test plants

The survival rate of *M. atropurpureum* (*V*₁) and *C. cajan* (*V*₂) were studied under different treatments / treatment combinations with respect of time period. From Table 18 and 19, it was observed that all the treated secondary plants showed better survival rate over the control. The survival percentage was highest in Treatment 3 (Secondary plants with entrapped microbial consortia and potential inoculated microorganism + Farmyard manure). The plant variety *C. cajan* (*V*₂) showed better survival rate as compared to *M. atropurpureum* (*V*₁). The survival rate was in increasing trend till 45 Days and after that it started decreasing in Control (*T₀*), Treatment 1 (Secondary plants with entrapped microbial consortia) and 2 (Secondary plants with both entrapped microbial consortia and potential inoculated microorganisms). However, survival rate started decreasing after 75 Days in Treatment 3 (Secondary plants with entrapped microbial consortia and potential inoculated microorganism + Farmyard manure).

Results of Analysis of variance (Table 18.1 and 19.1) revels that variation in survivality of *M. atropurpureum* (*V*₁) and *C. cajan* (*V*₂) due to different treatments and time period are significant \{(F^* = 236.6; F^* = 7.04) (F^* = 5.99; F^* = 7.78) at 5% level\}.
5.9.2 Growth of test plants

The growth of two varieties (*M. atropurpureum* and *C. cajan*) of secondary plants were studied and the effect of different treatments on plant height were observed with time period. From Table 20 and 21, it was observed that all the treated secondary plants showed better plant height over the control. The secondary plants of variety *M. atropurpureum* showed better growth performance than plant variety *C. cajan* with respect of plant height. The maximum height in plant variety *M. atropurpureum* (54.3 cm) and *C. cajan* (49.6 cm) was observed after 90 days of transplantation in Treatment 3 (Secondary plants with entrapped microbial consortia and potential inoculated microorganism + Farmyard manure).

Results of Analysis of variance (Table 20.1 and 21.1) revels that variation in growth (plant height) of *M. atropurpureum* (*V*1) and *C. cajan* (*V*2) due to different treatments and time period are significant \((F^* = 24.19; F^* = 17.33) \) (\(F^* = 24.99; F^* = 18.09\) at 5% level).

5.9.3 Pod yield of test plants

The average number of pods in two varieties (*M. atropurpureum* and *C. cajan*) of secondary plants were studied and the effect of different treatments on the average number of pods per plant per pots were observed with time period. From Table 22, it was observed that all the treated secondary plants produced better number of pods over the control. The highest (19.33 & 25.33) number of pods by the secondary plants (*M. atropurpureum* and *C. cajan*) were
produced in Treatment 3 (Secondary plants with entrapped microbial consortia and potential inoculated microorganism + Farmyard manure) while the lowest (1 & 1.3 Nos.) in controlled plants (V₁ and V₂) were produced after 90 days of transplantation.

Analysis of variance reveals that (Table 22.1 and 23.1) reveals that variation in number of pods per plant of *M. atropurpureum* (V₁) and *C. cajan* (V₂) due to different treatments and time period are significant \((F^* = 7.58; F^* = 5.19)\) \((F^* = 7.07; F^* = 5.15)\) at 5% level.

### 5.9.4 Nodule production

The average number of nodules in two varieties (*M. atropurpureum* and *C. cajan*) of secondary plants were studied after three months of transplantation. After harvesting, the total numbers of root nodules counted (Table 24) and it was observed that all the treated secondary plants (*M. atropurpureum* and *C. cajan*) produced better nodule number over the control. The average highest nodule numbers was observed by the secondary plants of variety *C. cajan* (18.7) than *M. atropurpureum* (16.33) in Treatment 3 (Secondary plants with entrapped microbial consortia and potential inoculated microorganism + Farmyard manure).

### 5.9.5 Estimation of available P, nitrogen, organic carbon and matter

Estimation of available P, nitrogen, organic carbon and matter of the rhizosphere soil of the transplanted secondary plants of two varieties (*M. atropurpureum* and *C. cajan*) of secondary plants were studied after three
months of transplantation. From Table 25, it was observed that the percent values of organic carbon, organic matter, available P and nitrogen contents in all the treated secondary plants were maximum while the control plants exhibited the lowest % of organic matter, organic carbon, nitrogen and available P. Soil organic matter is considered to be an indicator of total biomass production in restored sites. Investigation revealed that Treatment 3 (Secondary plants with entrapped microbial consortia and potential inoculated microorganism + Farmyard manure) produced an appreciably enhanced soil organic matter accumulation, with concentrations increasing from 5.6–27.3% in *M. atropurpureum* (V₁) and 5.27 – 28.9% in *C. cajan* (V₂).

After detail investigation, the study ended with some of the important findings which are briefly enumerated below:

i. Major limiting factors in Tirap coalmine spoils are high sand content, Poor water holding capacity, acute acidity and reduced nutrients availability (i.e. lower organic matter, nitrogen and phosphorus).

ii. Low pH and high concentration of sulphate in water of the study area are due to presence of free H₂SO₄ & ferric sulphate along with other salts.

iii. Old OBD soil samples of Tirap Colliery showed higher population of bacteria and fungi than the new OBD soil. The population of bacteria was higher than the fungal population. Highest number of gram (−) ve bacteria was recorded in overburden soil samples of Tirap Colliery followed by
phosphate solubilizing bacteria, denitrifying bacteria, asymbiotic N₂ fixing bacteria and nitrifying bacteria.

iv. The vegetation pattern of Tirap Colliery includes both naturally occurring plants and introduced plants in OBD soil. Most of the plants growing profusely in the degraded soil of Tirap Colliery are belonging to families like Poaceae, Mimosaceae and Apiaceae

v. A total of 8 genera of fungi viz; Aspergillus sp., Penicillium sp. Curvularia sp, Trichoderma sp., Fusarium sp., Mucor sp., Mycelia sp. and Rhizopus sp. were identified along with few species of AM Fungi viz Glomus and Acalospora. Glomus species was dominant and recorded highest number of spores in C. dactylon of Poaceae Family.

vi. A total of 13 groups including genus Rhizobia and Pseudomonas were identified along with other unidentified groups. The bacterial isolates showed good growth at pH (7 and 8) and Temperature (25 ⁰C and 30 ⁰C). However some of the bacterial isolates were also able to grow fairly at low pH (4.5).

vii. Among the two varieties of test plants viz; M. atropurpureum (V₁) and C. cajan (V₂), the test plants of variety C. cajan showed better survival rate as compared to M. atropurpureum. However, M. atropurpureum showed better growth performance than plant variety C. cajan with respect of plant height.

viii. The test plants of variety M. atropurpureum and C. cajan produced maximum number of pods and nodules over the control. The average...
highest numbers of pods and nodules were produced by the secondary plants of variety *C. cajan* and *M. atropurpureum* in Treatment 3 (Secondary plants with entrapped microbial consortia and potential inoculated microorganism + Farmyard manure).

ix. Rhizosphere soils of the transplanted secondary plants of variety *C. cajan* (*V₂*) showed better nutrient status than *M. atropurpureum* (*V₁*) with highest percentage of organic carbon, organic matter, available P and nitrogen contents in Treatment 3 (Secondary plants with entrapped microbial consortia and potential inoculated microorganism + Farmyard manure).